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Nutritional fish pathology

Morphological signs of nutrient deficiency and toxicity in farmed fish

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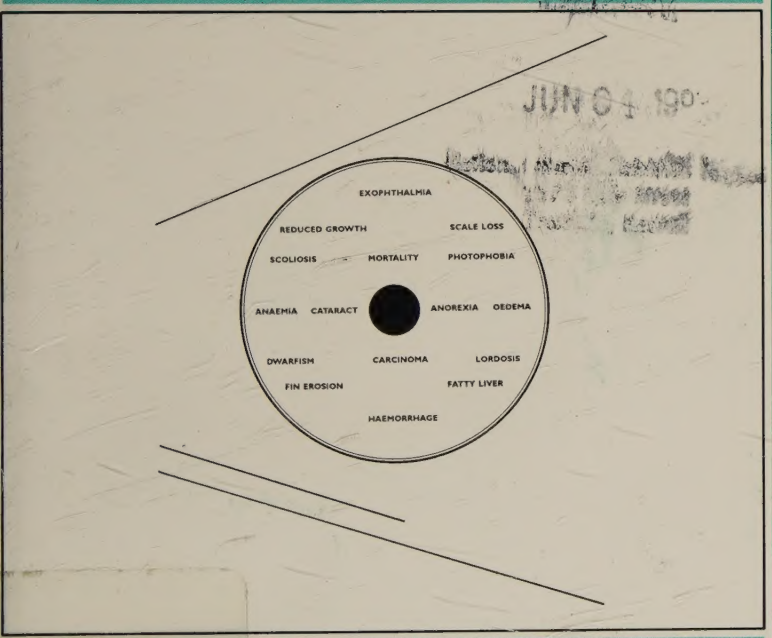

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Nutritional fish pathology

Morphological signs of nutrient deficiency and toxicity in farmed fish

by
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PREPARATION OF THIS DOCUMENT

This is the revised version of FAO/UNDP Field Document ADCP/REP/85/22, one of a series of reports periodically produced by the FAO Fishery Resources and Environment Division to help to meet the needs of aquaculture workers of Member Countries for synthesis of information in the field of aquaculture.

The report is based on the lecture text presented by the author to the trainees of the FAO International Training Course on Fish Disease Diagnosis organized by Project GCP/INT/526/JPN in Coyhaique, Chile from 23 November to 5 December 1992.

The information contained within the report is a synthesis of published data from a wide variety of sources relating to the morphological signs of dietary nutrient deficiency and toxicity in fish and, as such, constitutes a useful reference source and handbook for persons or parties engaged with the feeding of fish with nutritionally complete diets within clear-water intensive farming systems.

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ABSTRACT

The paper summarizes the major nutritional pathologies which have been reported in farmed fish. Morphological signs of nutrient deficiency and toxicity are presented and discussed under the following headings, 1) disorders in protein, lipid, mineral and vitamin nutrition, 2) endogenous anti-nutritional factors present in plant foodstuffs, and 3) adventitious toxic factors present in foodstuffs.

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1. INTRODUCTION

The first action that a farmer usually takes when faced with a population of sick or dying fish is to call a veterinarian or fish pathologist. If on examining the fish no recognisable disease organism is found (ie. bacteria, virus or parasite) then the incriminating finger is usually pointed toward the diet of the fish or to water quality as being the cause of the problem. However, on calling the local feed manufacturer the sales representative more often than not tries to place the blame on poor water quality or on other non-feed related factors. Throughout the above chain of events the farmer generally still remains in the dark, continues to suffer heavy fish mortalities, and is desperately in need of an unbiased opinion and solution to his or her problem. Although the above scenario is gradually improving with the development of modern disease diagnostic laboratories, nutritionally related 'disease' problems still remain a largely unchartered territory, veterinarians usually being too busy coping with their routine disease diagnostic duties, and nutritionists usually not being interested nor scientifically qualified to undertake pathological analyses or make judgements on pathology related issues. Clearly, fish pathologists and nutritionists will have to work in tandem in the future if rapid strides are to be made in the emerging and commercially important field of nutritional fish pathology; nutritional fish pathology being concerned with the study of those health disorders/ailments (often inappropriately referred to as 'diseases') which result from nutrient deficiencies or dietary imbalances.

In contrast to extensive and semi-intensive farming systems were fish obtain all or part of their dietary nutrient needs from naturally available pond food organisms, fish maintained under intensive culture systems rely totally on the provision of a nutritionally complete diet throughout their life cycle. For many farmed fish the development of commercial feed rations has proceeded despite the lack of reliable published information being available on their dietary nutrient requirements. In view of this paucity of information it is perhaps not surprising that dietary related pathologies have often arisen from specific nutrient deficiencies and imbalances under practical farming conditions. The aim of this paper is to summarize the major nutritional pathologies which have been reported within farmed fish.

2. DISORDERS IN PROTEIN NUTRITION

2.1 Dietary essential amino acid deficiency

Although all fish examined to date display reduced growth when fed essential amino acid (EAA) deficient diets, Table 1 shows the additional gross anatomical deficiency signs which have been reported under experimental conditions with juvenile fish fed rations deficient in one or more particular EAA's.

Table 1. Reported essential amino acid (EAA) deficiency signs in fish

Limiting EAA	Fish species	Deficiency signs [1/]
Lysine	Oncorhynchus mykiss	Dorsal/caudal fin erosion (1,2), increased mortality (2)
	Cyprinus carpio	Increased mortality (3)
Methionine	O. mykiss	Cataract (4,5,14)
	Salmo salar	Cataract (6)
Tryptophan	O. mykiss	Scoliosis [2/](7-10), lordosis [2/] (7,10), renal calcinosis (8), cataract (7,9), caudal fin erosion, decreased carcass lipid content (9); elevated Ca, Mag, Na and K carcass concentration (7)
	Oncorhynchus nerka	Scoliosis (11) Scoliosis (12,13), cataract (13) [3/]
	Oncorhynchus keta	Scoliosis (12)
	Oncorhynchus kisutch	
Miscellaneous	C. carpio	Increased mortality and incidence of lordosis observed with dietary deficiencies of leucine, isoleucine, lysine, arginine and histidine (3)

1/ 1-Walton, Cowey & Adron (1984), 2-Ketola (1983), 3-Mazid et al. (1978), 4-Walton, Cowey & Adron (1982), 5-Poston et al. (1977), 6-Barash, Poston & Rumsey (1982), 7-Walton et al. (1984), 8-Kloppel & Post (1975), 9-Poston & Rumsey (1983), 10-Shanks, Gahimer & Halver (1962), 11-Halver & Shanks (1960), 12-Akiyama et al. (1985), 13-Akiyama, Mori & Murai (1986), 14-Cowey et el. (1992)

2/ Curvature of the vertebral column

3/ Reported incidence of scoliosis and cataract increased with decreasing and increasing water temperature, respectively (13)

Under practical farming conditions dietary EAA deficiencies may arise from various routes, including:

- Poor feed formulation due to the use of disproportionate amounts of feed proteins with natural specific EAA deficiencies. Table 2 presents the chemical score and limiting EAAs of some selected food proteins available to the fish feed compounder. For the sake of comparison chemical scores have been calculated for individual protein sources with reference to the mean dietary EAA requirements of rainbow trout (O. mykiss) and common carp (C. carpio) as given by Ogino (1980). Compared to fish meal, which has a well balanced EAA profile, the majority of protein sources presented have amino acid imbalances which render them unsuitable as a sole source of dietary protein for fish. For example, the deficiency of methionine in plant proteins, yeast, meat and bone meal, blood meal, and hydrolysed feather meal; the deficiency of lysine in oilseeds, hydrolysed feather meal and algae; the deficiency of threonine in some oilseeds and pulses; and the deficiency of tryptophan in fish silage. It is clear from the above that during feed formulation special care must be given to the choice of feedstuffs used so that the desired overall dietary EAA profile is obtained.

  Dietary imbalances may also arise from the presence of disproportionate levels of specific amino acids; including leucine/isoleucine antagonisms, and to a lesser extent arginine/lysine and cystine/methionine antagonisms. For example, blood meal is a rich source of valine, leucine and histidine, but is a very poor source of methionine and isoleucine. However, in view of the antagonistic effect of excess leucine on isoleucine, animals fed high dietary levels of blood meal suffer from an isoleucine deficiency caused by an excess of dietary leucine (Taylor, Cole & Lewis, 1977). Although similar antagonisms have also been reported for cystine/methionine (use of hydrolysed feather meal; Ichhponani & Lodhi, 1976) and arginine/lysine (Harper, Benevenga & Wohlhueter, 1970) in terrestrial farm animals, they have not been reported to occur in fish fed synthetic amino acid diet combinations (Robinson, Wilson & Poe, 1981).

- Dietary EAA deficiencies may arise from excessive heat treatment of feed proteins during feed manufacture. For example during fish meal manufacture excessive heat treatment has been shown to markedly reduce protein digestibility and biological value due to the destruction of amino acids by oxidation or through the formation of linkages between individual amino acids which are more resistant to digestion (McCallum & Higgs, 1989; Pike, Andorsdottir & Mundheim, 1990). The free epsilon amino groups of lysine are particularly susceptible to heat damage, forming addition compounds with non-protein molecules (reducing

sugars) present in the foodstuff (Cockerell, Francis & Halliday, 1972). In addition to decreased EAA availability, toxic substances such as gizzerosine (2-amino-9-(4-imidazolyl)-7-azanonanoic acid) may also be produced from heat treated fish meals containing free histidine and histamine (Okayaki et al., 1983; Watanabe et al. 1987). Although gizzerosine is reported to cause gizzard erosion (GE) in chicks, toxicological signs of rainbow trout fed comparable heat treated whole fish meals containing high levels of histidine and histamine included decreased stomach wall thickness, pycanosis and necrosis of gastric gland cells (Watanabe et al. 1987).

Table 2. Chemical score and limiting essential amino acids of selected protein sources 1/

Feedstuff	Ref2/	THR	VAL	MET	CYS	IIS	LEU	PHE	TYR	LYS	HIS	ARG	TRP	LAA3/
Chick pea	1	64*	89	63*	104	119	110	113	86	72	100	166	129	Met
Mung bean	1	59*	110	54*	48*	127	121	124	94	79	114	123	123	Cys
Cow pea	1	65*	103	61*	59*	116	116	116	100	75	127	134	129	Cys
Yellow lupin	2	66*	81	20*	126	117	125	85	94	64*	117	192	135	Met
Lima bean	2	84	110	57*	74	135	118	125	106	72	112	98	106	Met
Broad bean	3	77	103	30*	41*	115	118	98	118	77	98	160	118	Met
Haricot bean	1	80	103	43*	67*	120	121	118	83	92	127	104	129	Met
Safflower	2	68*	125	63*	141	111	99	101	100	43*	121	181	118	Lys
Crambe	2	98	121	67*	218	117	104	83	86	66*	104	111	200	Lys
Palm kernel	2	62*	113	94	133	95	89	72	78	41*	98	225	311	Lys
Cottonseed	2	65*	102	52*	118	92	94	122	89	52*	117	205	141	Lys
Sunflower	2	65*	124	83	137	115	104	109	91	42*	119	159	165	Lys

Linseed	2	71	122	93	156	111	90	105	92	43*	100	174	182	Lys
Sesame	2	58*	98	109	148	91	105	86	114	33*	114	211	153	Lys
Coconut	4	65*	114	61*	96	115	112	95	92	37*	81	217	123	Lys
Groundnut	4	55*	99	39*	133	117	100	107	117	53*	100	196	141	Met
Rapeseed	4	93	118	83	70	113	116	94	77	74	131	112	159	Cys
Soybean	4	74	101	46*	130	128	115	105	97	76	106	123	176	Met
Potato protein meal	5	89	125	63*	96	128	120	112	149	74	73	73	118	Met
Leaf protein meal	6	84	127	57*	56*	112	120	122	129	71	90	96	141	Cys
Spirulina maxima	2	87	136	2*	30*	159	118	105	123	55*	75	111	165	Cys
Bakers dried yeast	4	93	116	63*	86	139	112	91	108	86	106	89	141	Met
Torulopsis utilis	4	94	118	54*	81	144	98	137	117	84	104	86	118	Met

Bacterial SCP	7	97	134	89	59*	115	107	115	138	71	83	84	118	Cys
Whole hen's egg	8	77	125	100	130	132	109	97	98	78	92	96	135	Thr
Fish muscle	9	83	98	98	85	108	110	80	117	101	121	97	135	Phe
Fish meal (herring)	4	76	127	109	78	117	107	80	95	89	96	111	123	Thr
Fish meal (white)	4	81	106	104	93	121	109	81	94	90	94	116	129	Thr
Fish protein concentr.	2	83	110	118	63*	127	109	85	103	92	90	95	153	Cys
Fish silage	10	98	122	72	72	101	129	120	94	98	121	108	59*	Trp
Whole shrimp meal	2	83	97	109	85	112	106	95	105	86	73	134	106	His
Meat and bone meal	4	77	128	59*	89	109	113	88	60*	86	100	150	88	Met
Blood meal	4	69*	158	33*	52*	24*	162	124	69*	89	214	62*	123	Ils
Liver meal	2	76	135	72	89	105	121	109	106	71	98	105	153	Lys

Poultry byproduct meal	4	76	125	81	141	132	123	80	60*	71	87	134	112	Tyr
Hydrolyzed feathers	4	91	164	24*	289	131	124	78	86	33*	50*	147	76	Met
Earthworm meal	11	107	99	106	52*	112	124	84	108	79	125	98	82	Cys
House fly larvae	12	75	103	72	52*	96	90	128	218	77	127	82	147	Cys

1/ Scores based on comparison with the mean essential amino acid requirements of rainbow trout and common carp (Ogino, 1980). Mean EAA requirement (expressed as % of total EAA) being: threonine 10.6, valine 9.5, methionine 5.4, cystine 2.7, isoleucine 7.5, leucine 13.5, phenylalanine 9.5, tyrosine 6.5, lysine 16.8, histidine 4.8, arginine 11.6 and tryptophan 1.7

2/ Source: 1-Kay (1979), 2-Gohl (1980), 3-Bolton & Blair (1977), 4-National Research Council (1983), 5-Tunnel Avebe Starches Ltd (UK), 6-Cowey et al. (1971), 7-Methanobacter methylotrophus Unpublished data, 8-Cowey & Sargent (1972), 9-Connell & Howgate (1959), 10-Jackson, Kerr & Cowey (1984), 11- Tacon, Stafford & Edwards (1983), 12-Spinelli (1980)

* Limiting essential amino acids (present below 30% mean fish requirement)

- Dietary EAA deficiencies may arise from the chemical treatment of feed proteins with acids (silage production) or alkalies, due to the loss of free tryptophan and lysine/cystine respectively (Kies, 1981).

- Dietary EAA deficiencies may arise from the leaching of free and protein bound amino acids into the water. For example, Grabner, Wieser & Lackner (1981), reported the loss, through leaching, of almost all the free and about one-third of the free plus protein bound amino acids from frozen or freeze-dried zooplankton (Artemia salina and Moina spp.) after a ten minute immersion period at 9°C. A similar rapid leakage of free amino acids (FAA) has also been observed from frozen Daphnia upon thawing; 23.1% leakage of all FAA during five minutes in water after thawing (Holm & Walther, 1988).

2.2 Toxic amino acids

Nutritional pathologies may arise from the consumption of feed proteins containing toxic amino acids or their derivatives. Feed proteins containing toxic amino acids which have been reported to have a negative effect on fish growth and feed efficiency (including eventual fish death) include the plant legumes Leucaena leucocephala (toxic nonprotein amino acid - mimosine; Jackson, Capper & Matty, 1982; Wee & Wang, 1987) and Sesbania grandiflora and Canavalia ensiformis (toxic amino acid - L-canavanine; Martinez-Palacios et al. 1988, Olvera et al. .1988).

In addition to the non-essential amino acids, certain EAA (ie. leucine) have also been reported to exert a toxic effect in fish when present in dietary excesses (Hughes, Rumsey & Nesheim, 1984; Robinson, Poe & Wilson, 1984). For example, the reported toxicity signs for a dietary excess of leucine (13.4% of diet) in rainbow trout (O. mykiss) included scoliosis, deformed opercula, scale deformities, scale loss, and spongiosis of epidermal cells (Choo et al. 1991).

3. DISORDERS IN LIPID NUTRITION

3.1 Dietary essential fatty acid deficiency

All fish examined to date display reduced growth and poor feed efficiency when fed experimental diets deficient in essential fatty acids (EFA). Table 3 shows the additional gross anatomical deficiency signs which have been reported with juvenile fish fed EFA deficient diets. In general dietary EFA deficiencies result from poor feed formulation or from the use of EFA deficient live food organisms.

Table 3. Reported essential fatty acid (EFA) deficiency signs in fish

Fish species	Deficiency signs [1/]
O. mykiss	Increased mortality, elevated muscle water content, increased susceptibility to caudal fin erosion by Flexebacterium sp., fainting or shock syndrome, decreased haemoglobin and red blood cell volume (1), fatty infiltration and degeneration of liver, swollen pale liver (1,2), reduced spawning efficiency (low hatching and larval survival rate, 3)
Oncorhynchus kisutch	Swollen pale liver, increased hepatosamtic index (fatty liver), high mortality (2)
Oncorhynchus keta	Swollen pale liver, increased hepatosamtic index (fatty liver), high mortality (2)
C. carpio	Increased mortality (4), fatty liver (7)
Anguilla japonica	Increased mortality (6)
Oreochromis niloticus	Swollen pale liver, fatty liver (7)
Pagrus major	Reduced spawning efficiency (decreased hatching and survival rates (3), reduced appetite and growth, increased liver lipid content, high mortality (12)
Lates calcarifer	Reduced growth and feed efficiency, reddening of fins (8)
Scophthalmus maximus	Increased mortality, reduced growth, degeneration of gill epithelium (9)
Ctenopharyngodon idella	Reduced growth and feed efficiency, swollen pale liver, increased mortality, lordosis, shock syndrome (10)
Pseudocaranx dentex	Reduced appetite and swimming activity, reduced growth and feed efficiency, high mortality (11)
Coregonus lavaretus	Reduced growth, feed efficiency and survival rate, swollen pale liver (13,14)

1/ 1-Castell et al. (1972), 2-Takeuchi & Watanabe (1982), 3-Watanabe (1982), 4-Takeuchi & Watanabe (1977), 5-Farkas et al. (1977), 6-Takeuchi et al. (1980), 7-Takeuchi, Satoh & Watanabe (1983), 8-Wanakowat et al. (1991), 9-Bell et al. (1985), 10-Takeuchi et al. (1991), 11-Watanabe et al. (1989), 12-Takeuchi et al. (1990), 13-Watanabe et al. (1989a), 14-Thongrod et al. (1989)

3.2 Dietary essential fatty acid toxicity

Under laboratory conditions it has been found that a dietary excess of EFA may exert a negative effect on fish growth and feed efficiency (rainbow trout - Yu & Sinnhuber, 1976; Takeuchi & Watanabe, 1979; coho salmon - Yu & Sinnhuber, 1979).

3.3 Toxic non-essential fatty acids

Cyclopropenoic acid is a toxic fatty acid found in the lipid fraction of cottonseed products. Experimentally, cyclopropenoic acid has been shown to reduce growth rate in rainbow trout and to act as a potent synergist for the carcinogenity of aflatoxins (Lee & Sinnhuber, 1972; Hendricks et al. 1980). Other pathologies observed with trout include extreme liver damage (liver is pale in colour) with increased glycogen deposition and decreased protein content, and a decrease in activity of several key enzymes (Roehm et al. 1970, Taylor, Montgomery & Lee, 1973).

3.4 Oxidation of dietary lipids

In the absence of suitable antioxidant protection lipids rich in polyunsaturated fatty acids (PUFA, including EFA) are highly prone to auto-oxidation on exposure to atmospheric oxygen. Under these conditions, the nutritional benefit of EFA in fact becomes deleterious to the health of the fish. Feedstuffs rich in PUFA which are particularly susceptible to lipid oxidative damage (oxidative rancidity) include fish oils, fish meal, rice bran and expeller oilseed cakes containing little or no natural antioxidant activity. During the process of lipid auto-oxidation chemical degradation products are formed, including free radicals, peroxides, hydroperoxides, aldehydes and ketones, which in turn react with other dietary ingredients (vitamins, proteins and other lipids) reducing their biological value and availability during digestion. At present oxidative rancidity is believed to be one of the most deteriorative changes which occurs in stored feedstuffs (Cockerell, Francis & Halliday, 1972; Chow, 1980). Table 4 summarizes the major anatomical pathological signs which have been reported in fish fed rations containing oxidized fish/plant oils with no antioxidant (vitamin E) protection.

With the exception of the study of Soliman, Roberts & Jauncey (1983) with Oreochromis niloticus, the pathological effects of oxidized lipids have been shown to be prevented by dietary supplementation with dl-alpha tocopherol acetate (vitamin E). During the six weeks feeding trial of Soliman, Roberts & Jauncey (1983) the dietary supplementation of vitamin E to an oxidized fish oil diet only prevented the occurrence of lordosis. Although no vitamin E analyses were performed on diet or fish tissue at the end of the experiment, in contrast to previous studies with fast growing tropical fish these authors also reported no pathological deficiency signs in fish fed diets containing fresh lipid with no dietary vitamin E supplementation. Clearly, longer term studies will be required to confirm these findings.

In the absence of suitable antioxidant protection the rate of lipid auto-oxidation in stored feedstuffs has been found to increase in the presence of lipoxidase (present in raw soybeans), haeme compounds (myoglobin and haemoglobin are pro-oxidants present in meat meals and fishmeals), peroxides (product of lipid auto-oxidation), light

(UV - formation of singlet oxygen/free radicals), increased temperature (reaction rate), and trace elements (Fe and Cu have been found to accelerate lipid oxidation by direct electron transfer in redox reactions, whereas Zn induces the breakdown of hydroperoxides to free radicals (ADCP, 1983).

Table 4. Reported pathological effects of oxidized fish oil in fish

Fish species	Pathological effects [1/]
O. niloticus	Marked congestion, with some haemorrhage, in dermal vessels around snout and at bases of pectoral/dorsal fins, lordosis, exophthalmia, abdominal swelling (oedema), cataract, orbital collapse, darkening of liver, marked distension of bile duct, steatitis of all abdominal fat bearing tissue, deposits of intracellular ceroid in liver, spleen, kidney and choroid, increased mortality (1)
C. carpio	Poor growth, loss of appetite, muscular dystrophy, high mortality, reduced absorption of dietary lipids (3-5)
Ictalurus punctatus	Poor growth, poor feed efficiency, increased mortality, exudative diathesis (increased permeability of blood capillaries), muscular dystrophy, depigmentation, fatty livers (6)
Seriola quinqueradiata	Reduced growth, swollen liver, decreased lipid deposition (7), anorexia (loss of appetite), leaning of dorsal muscle, muscular dystrophy (8)
Oncorhynchus tshawytscha	Dark body colouring, anaemia, lethargy, brown-yellow pigmented liver (ceroid deposition), abnormal kidney and evidence of gill clubbing (2)
O. mykiss	Reduced growth (9,10), poor feed efficiency (9), microcytic anaemia (10,11), reduced haematocrit and haemoglobin content, liver lipoid degeneration (ceroid accumulation; 10-11,13), severe muscle damage (9), increased mortality and erythrocyte fragility (9,11,12)
Salmo salar	Reduced growth, increased mortality (14)
O. kisutch	Reduced growth (14,15), increased mortality (14), reduced feed efficiency (15)

1/ 1-Soliman, Roberts & Jauncey (1983), 2-Fowler & Banks (1969), 3-Watanabe & Hashimoto (1968), 4-Hashimoto et al. (1966), 5-Hata & Kaneda (1980), 6-Murai & Andrews (1974), 7-Park (1978), 8-Sakaguchi & Hamaguchi (1969), 9-Cowey et al. (1984), 10-Smith (1979), 11-Moccia et al. (1984), 12-Hung, Cho & Slinger (1981), 13-Rehulka (1990), 14-Ketola, Smith & Kindschi (1989), 15-Forster et al. (1988)

4. DISORDERS IN MINERAL NUTRITION

4.1 Dietary essential mineral deficiency

Table 5 shows the major anatomical deficiency signs which have been reported in fish fed rations deficient in one or more essential minerals. Despite the presence of macro and micro (trace) elements in virtually all raw ingredients commonly used for fish feeding (Tacon & De Silva, 1983) and the ability of fish to absorb certain trace elements from the surrounding water, mineral deficiencies may arise under intensive culture conditions through:

- The absence of a specific macro or trace mineral premix within the diet. For details of specific mineral premix formulations and dietary recommendations see NRC (1983) and Davis & Gatlin (1991).

- Reduced mineral bioavailability through dietary imbalances. The availability and utilization of dietary trace elements in fish is dependent upon the dietary source and form of the element ingested, the adequacy of stores within the body, interactions with other mineral elements present in the gastro-intestinal tract and within the body tissues (antagonisms), and finally by element interactions with other dietary ingredients or their metabolites (ie. vitamins, fibre or phytic acid). For example, Table 6 shows the relative availabilities or apparent absorption efficiency of various forms or sources of dietary phosphorus for channel catfish (I. punctatus), common carp (C. carpio) and rainbow trout (O. mykiss). In general, phosphorus bioavailability has been found to be higher in brown low-ash fish meals than in high-ash white fish meals (Lall & Keith, 1991, Watanabe, Satoh & Takeuchi, 1988).

For certain fish species the availability and absorption of phosphorus and other major elements (calcium) from fish meal and meat and bone meal is further complicated by the absence of an acid-secreting stomach, which is essential for normal bone solubilization. For stomachless fish species soluble monobasic inorganic salts or bioavailable organic salts must therefore be provided in the diet. Conversely, within plant proteins a large proportion of phosphorus is present as organically bound phytates. Not only is phytic acid phosphorus largely biologically unavailable, but phytic acid also has the capacity to chelate other trace elements (ie. iron, copper, zinc, cobalt, molybdenum) and by so doing may render them biologically unavailable to the fish during digestion (Spinelli, 1980; Lovell, 1989; Hossain & Jauncey, 1991). For example, in channel catfish (I. punctatus) dietary phytate has been shown to reduce the bioavailability of zinc, especially in the presence of high dietary calcium (Gatlin & Phillips, 1989). Furthermore, high dietary phytate levels (2.2%) have also been reported to have a negative effect on fish growth and feed efficiency in channel catfish (Satoh, Poe & Wilson, 1989).

Under practical farming conditions mineral deficiency signs often arise from a dietary imbalance of calcium; due to the antagonistic effect of excess dietary calcium on the absorption of phosphorus (Nakamura, 1982) and the trace elements zinc, iron and manganese (Lall, 1979). For example, the bioavailability of zinc and to a lesser extent manganese and magnesium, within white fish meal has been found to be much lower than that contained in brown fish meal (which has a much lower ash and calcium content; Ketola, 1978; Watanabe, Takeuchi & Ogino, 1980; Satoh, Takeuchi & Watanabe, 1987a, 1987b, 1991). Similarly, increasing levels of dietary calcium phosphate ($Ca_3(PO_4)_2$ and $Ca(H_2PO_4)_2$) was found to have a inhibitory effect on zinc bioavailability in rainbow trout, inducing short body dwarfism or eye cataracts (Satoh et al. 1987, 1991). It is perhaps not surprising therefore that rainbow trout, chum salmon and common carp fed on diets in which white fish meal was used without a trace element supplement that overt trace element deficiency signs arise, including reduced growth, short body dwarfism and cataracts (Watanabe, Takeuchi & Ogino, 1980; Satoh et al. 1983, 1983a; Yamamoto et al. 1983; Watanabe, Satoh & Takeuchi, 1988). Furthermore, recent trials concerning the dietary zinc requirements of channel catfish (I. punctatus)swim-up fry in soft and hard water appear indicate that environmental calcium interacts with dietary zinc and may also fry growth and survival (Scarpa & Gatlin, 1992). However, the recent trials of Satoh et al. (1991) also suggest that high dietary intakes of phosphorus (1.8% of diet) also has a negative effect on growth and zinc availability, thus indicating the importance of the balance between dietary calcium and phosphorus; the best growth reported for rainbow trout fed calcium and phosphorus in equal proportions.

4.2 Dietary mineral toxicity

A major hazard which may be associated with the use of unconventional dietary feed ingredients is the presence of heavy metal contaminants including the accumulative elements copper, lead, cadmium, mercury, arsenic, fluoride, selenium, molybdenum and vanadium. For example, contamination with copper may arise from products fermented within copper lined vessels (brewery by-products), or within pig and poultry excreta from the use of copper based growth stimulants and anti-fungal agents. Other feed ingredients which may contain metal contaminants include: poultry waste - arsenic; paper pulp waste -lead; fish meals - mercury, selenium, arsenic, cadmium and lead; poultry by-product meals - zinc; hydrolysed feather meals - zinc; shellfish - zinc, cadmium; seleniferous accumulating plants of the genera Astragalus and Machaeranthera, or cereals grown in seleniferous soils - selenium; krill meal - fluoride. Table 7 lists the major toxicity signs which have been reported in fish under laboratory conditions.

Table 5. Reported essential mineral deficiency signs in fish

Element/fish sp.	Deficiency signs [1/]
PHOSPHORUS (P)	
C. carpio	Reduced growth, poor feed efficiency (1,2), bone demineralization, skeletal deformity, abnormal calcification of ribs and the soft rays of the pectoral fin (1), cranial deformity (1,3), increased visceral fat (4)
I. punctatus	Reduced growth, poor feed efficiency (5), bone demineralization (5,6)
P. major	Reduced growth, poor feed efficiency, bone demineralization, increased muscle, liver and vertebrae lipid content (7), curved and enlarged spongy vertebrae (8), decreased liver glycogen (9)
A. japonica	Anorexia, reduced growth (10)
O. mykiss	Reduced growth, poor feed efficiency, bone demineralization (13,14)
S. salar	Reduced growth, poor feed efficiency, bone demineralization (13,14)
CALCIUM (Ca)	
I. punctatus	Reduced growth, low carcass ash, Ca and P content (fed vitamin D deficient diet, 6)
O. mykiss	Anorexia, poor growth and feed efficiency (15)
A. japonica	Anorexia, ppor growth and feed efficiency (16)
P. major	Anorexia, poor growth and feed efficiency (17)
POTASSIUM (K)	
O. tshawytscha	Reduced growth and feed efficiency, anorexia, convulsions, tetany, death (42)
MAGNESIUM (Mg)	
C. carpio	Reduced growth (11,18), sluggishness, anorexia, convulsions, high mortality, reduced bone magnesium content (11), cataracts (18)
I. punctatus	Anorexia, reduced growth, sluggishness, muscle flaccidity, high mortality, depressed Mg content in body and blood serum/bone (19)
A. japonica	Anorexia, reduced growth (20)
O. mykiss	Reduced growth (21-24), anorexia (22-23), cataract (25), sluggishness, calcinosis of kidney (21-22), increased mortality, vertebral curvature, degeneration of muscle fibres and epithelial cells of pyloric caeca and gill filaments (23), reduced bone ash, Mg and elevated Ca content (24)

Poecilia reticulata	Reduced growth and feed efficiency, high mortality (43)
IRON (Fe)	
General	Hypochromic microcytic anaemia (C. carpio-26, P. major-27, Salvelinus fontinalis-28, A. japonica-20)
Element/fish sp.	**Deficiency signs** [1/]
ZINC (Zn)	
I. punctatus	Reduced growth and appetite, depressed bone Ca and Zn content, and serum Zn (29)
C. carpio	Reduced growth (18,30), cataracts (18), loss of appetite, high mortality, erosion of fins and skin, elevated tissue concentration of Fe and Cu in intestine and hepatopancreas (30)
O. mykiss	Reduced growth (25,31-32,44), increased mortality (31-32), cataracts (25,31,44), short body dwarfism (25,44), fin erosion (31)
MANGANESE (Mn)	
O. mossambicus	Reduced growth and appetite, loss of equilibrium, mortality (33)
C. carpio	Reduced growth (34,18), short body dwarfism, cataracts (18)
O. mykiss	Cataracts (25,35), reduced growth, short body dwarfism (34,35), abnormal tail growth (34)
COPPER (Cu)	
C. carpio	Reduced growth (34,18), cataracts (18)
SELENIUM (Se)	
S. salar	Increased mortality, muscular dystrophy, depressed glutathione peroxidase (enzyme) activity (36), reduced growth (37)
C. carpio	Reduced growth (18,38), cataracts (18), anaemia (38)
I. punctatus	Reduced growth (39)

Table 5. Reported essential mineral deficiency signs (continuation)

IODINE	
Salmonids	Thyroid hyperplasia (goitre, 40-41)

1/ 1-Ogino & Takeda (1976), 2-Yone & Toshima (1979), 3-Ogino et al. (1979), 4-Takeuchi & Nakazoe (1981), 5-Andrews, Murai & Campbell (1973), 6-Lovell & Li (1978), 7-Sakamoto & Yone (1980), 8-Sakamoto & Yone (1979), 9-Sakamoto & Yone (1978), 10-Arai, Nose & Kawatsu (1974), 11-Ogino & Chiou (1976), 12-Ogino & Takeda (1978), 13-Ketola (1975), 14-Lall & Bishop (1977), 15-Arai et al. (1975), 16-Arai, Nose & Hashimoto (1975), 17-Sakamoto & Yone (1973), 18-Satoh et al. (1983), 19-Gatlin et al. (1982), 20-Nose & Arai (1979), 21-Cowey et al (1977), 22-Knox, Cowey & Adron (1981), 23-Ogino, Takashima & Chiou (1978), 24-Knox, Cowey & Adron (1983), 25-Satoh et al. (1983a), 26-Sakamoto & Yone (1978a), 27-Sakamoto & Yone (1978b), 28-Kawatsu (1972), 29-Gatlin & Wilson (1983), 30-Ogino & Yang (1979), 31-Ogino & Yang (1978), 32-Wekell, Shearer & Houle (1983), 33-Ishak & Dollar (1968), 34-Ogino & Yang (1980), 35-Yamamoto et al (1983), 36-Poston, Combs & Leibovitz (1976), 37-Bell et al. (1987), 38-Lall (1979), 39-Gatlin & Wilson (1984), 40-Woodall & La Roche (1964), 41-NRC (1983), 42-Shearer (1988), 43-Shim & Ng (1988), 44-Satoh, Takeuchi & Watanabe (1987b)

Table 6. Reported dietary phosphorus availability (%) in selected fish [1/]

Dietary source	Channel catfish	Common carp	Rainbow trout
PHOSPHATES (PO_4)			
Sodium PO_4, monobasic	90	94	98
Potassium PO_4, mono	-	94	98
Calcium PO_4, monobasic	94	94	94
Calcium PO_4, dibasic	65	46	71
Calcium PO_4, tribasic	-	13	64
FISH MEALS			
White fishmeal	-	0-18	66
Brown fishmeal	-	24	74
Anchovy fishmeal	40	-	-
Menhaden fishmeal	39	-	-
PROTEIN SOURCES			
Egg albumin	71	-	-
Casein	90	97	90
Brewers yeast	-	93	91
PLANT PRODUCTS			
Rice bran	-	25	19
Wheat germ	-	57	58
Wheat middlings	28	-	-
Corn, ground	25	-	-
Soybean meal, + hulls	50	-	-
Soybean meal, dehulled	29-54	-	-
Phytate	0	8-38	0-19

1/ Source: NRC (1983)

Table 7. Reported dietary mineral toxicity signs in fish

Element	Fish species	Toxicity signs 1/
Zinc	C. carpio	Reduced growth (dietary level above 300mg/kg; 1)
Copper	I. punctatus	Reduced growth (dietary level above 15mg/kg; 2)
Selenium	O. mykiss	Reduced growth and feed efficiency, high mortality (dietary level above 13mg/kg (3,4), nephrocalcinosis (4,5)
	I. punctatus	Reduced growth (dietary levels above 15mg/kg; 6)
Cadmium	O. mykiss/C. carpio	Scoliosis, hyperactivity, decreased bone calcium content (7-10)
Lead	O. mykiss	Scoliosis, lordosis, black tail, anaemia, degeneration of caudal fin (11)
Chromium	O. mykiss	Reduced growth and feed efficiency (12)

1/ 1-Jeng & Sun (1981), 2-Murai, Andrews & Smith (1981), 3-Hilton, Hodson & Slinger (1980), 4-Hicks, Hilton & Ferguson (1984), 5-Hilton & Hodson (1983), 6-Gatlin & Wilson (1984), 7-Koyama & Itazawa (1977), 8-Koyama & Itazawa (1977a), 9-Koyama & Itazawa (1979), 10-Roch & Maly (1979), 11-Johansson-Sjobeck & Larsson (1979), Tacon & Beveridge (1982)

5. DISORDERS IN VITAMIN NUTRITION

5.1 Dietary vitamin deficiency

Table 8 shows the major gross anatomical deficiency signs which have been reported in fish fed vitamin deficient diets.

Table 8. Reported dietary vitamin deficiency signs in fish

Vitamin/fish sp.	Deficiency signs [1]
RIBOFLAVIN (vitamin B2)	
Salmonids	Anorexia, poor growth, corneal vascularisation, cloudy lens, snout erosion, spinal deformities, increased mortality rate, severe fin erosion, fin haemorrhage, rapid opercular movement, apparent muscular weakness, light or dark pigmentation, striated constrictions of abdominal wall, photophobia, incoordination, lethargy, anaemia (1-10)
C. carpio	Anorexia, poor growth, high mortality rate, haemorrhage of skin and fins, nervousness, photophobia (11-12)
I. punctatus	Short body dwarfism, anorexia, poor growth, cataract (13-14)
P. major	Poor growth (15)
A. anguilla	Fin haemorrhage, photophobia, poor growth, anorexia, lethargy (16)
Clarias batrachus	Anorexia, poor growth, haemorrhage of skin and fins, increased mortality rate, eroded barbels, oedema, fading of body colour, lethargy, pale gills and liver, cloudy lens (17)
Lates calcarifer	Sluggishness, photophobia, cataracts, stunted body, reduced growth, feed efficiency and survival, dark colouration (18)
O. mossambicus x urolepis hornorum)	Anorexia, reduced growth, light colouration, nervous symptoms, mortality, dwarfism, cataract (19)

1-Mclaren et al. (1947), 2-Phillips & Brockway (1957), 3-Halver (1957), 4-Kitamura et al. (1967), 5-Poston et al. 1977, 6-Takeuchi et al. (1980), 7-Hughes, Rumsey & Nickum (1981), 8-Woodward (1982), 9-Woodward (1985), 10-Amezaga & Knox (1990), 11-Aoe et al. (1967), 12-Ogino (1967), 13-Dupree (1966), 14-Murai & Andrews (1978a), 15-Yone (1975), 16-Arai, Nose & Hashimoto (1972), 17-Butthep, Sitasit & Boonyaratpalin (1985), 18-Boonyaratpalin & Wanakowat (1991), 19-Lim, Leamaster & Brock (1991)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
PANTOTHENIC ACID	
Salmonids	Anorexia, reduced growth, gill necrosis/clubbing, anaemia, mucous covered gills, sluggish activity, opercules distended (1-6)
C. carpio	Anorexia, reduced growth, sluggishness, anaemia, skin haemorrhage, exophthalmia (7)
I. punctatus	Anorexia, clubbed gills, eroded skin, lower jaws and head, anaemia (8-10)
P. major	Poor growth, mortality (11-12)
A. anguilla	Poor growth, abnormal swimming behaviour, skin lesions (13)
C. batrachus	Anorexia, reduced growth, high mortality, clubbed gills, haemorrhage under the skin, fragile fins, oedema, eroded barbels, rapid breathing, swelling at base of pectoral fins, pale gills and liver (14)
Cichlasoma urophthalmus	Anorexia, high mortality, rapid breathing, dark colouration, distended operculae, slight exophthalmia, haemorrhage on fins and head (eyes), inter lamellar lesions (including fusion of adjacent filaments; 15)
L. calcarifer	Anorexia, reduced feed efficiency, weight gain and survival, dark colouration, abnormal swimming, haemorrhagic operculum, eroded pelvic fin, clubbed gills (16)

1/ 1-McLaren et al. (1947), 2-Phillips & Brockway (1957), 3-Halver (1957), 4-Kitamura et al. (1967), 5-Coates & Halver (1958), 6- Masumoto, Hardy & Stickney (1991), 7-Ogino (1967), 8-Dupree (1966), 9-Murai & Andrews (1979), 10-Wilson, Bowser & Poe (1983), 11-Yone (1975), 12-Yano et al. (1988), 13-Arai, Nose & Hashimoto (1972), 14-Butthep, Sitasit & Boonyaratpalin (1985), 15-Chavez de Martinez, Escobar & Olvera-Novoa (1990), 16-Boonyaratpalin & Wanakowat (1991)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
NIACIN (nicotinic acid)	
Salmonids	Anorexia, poor growth, reduced feed efficiency, dark colouration, erratic swimming, muscle spasms while resting, oedema of stomach, susceptibility to sunburn (1-5)
C. carpio	Skin haemorrhage, high mortality (6)
I. punctatus	Haemorrhage and lesions of skin/fin, deformed jaws, anaemia, exophthalmia, high mortality (7-8)
P. major	Poor growth (9)
A. japonica	Haemorrhage and skin lesions, reduced growth, ataxia (abnormal swimming), dark colouration (10)
C. batrachus	Anorexia, reduced growth, muscle spasms, loss of equilibrium, whirling, lethargy, haemorrhage under the skin and fins, slight exophthalmia, high mortality, erratic swimming (11)
BIOTIN	
Salmonids	Anorexia, reduced growth, increased mortality, poor feed efficiency, blue-slime disease (brook trout only), lesions in the colon, muscle atrophy, spastic convulsions, thick gill lamellae, pale gills (2-3,12-18)
C. carpio	Reduced growth and activity (19-20)
I. punctatus	Depigmentation, anaemia, anorexia, reduced growth, hypersensitivity (21-22)
A. japonica	Poor growth, dark colouration, abnormal swimming behaviour (10)

1/ 1-McLaren et al. (1947), 2-Phillips & Brockway. (1957), 3-Halver (1957), 4-Poston & Di Lorenzo (1973), 5-Poston & Wolfe (1985), 6-Aoe et al. (1967), 7-Dupree (1966), 8-Andrews & Murai (1978), 9-Yone (1975), 10-Arai, Nose & Hashimoto (1972), 11-Butthep, Sitasit & Boonyaratpalin (1985), 12-Kitamura et al. (1967), 13-Coates & Halver (1958), 14-Walton et al. (1984), 15-Poston & McCartney (1974), 16-Poston (1976), 17- Castledine et al. (1978), 18-Poston & Page (1982), 19-Ogino et al. (1970), 20-Gunther & Meyer-Burgdorff (1990), 21-Robinson & Lovell (1978), 22-Lovell & Buston (1984)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
THIAMINE (vitamin B1)	
Salmonids	Anorexia, poor growth, nervous disorders, increased sensitivity to shock by physical blow to container or from light flashes (1-5)
C. carpio	Fin haemorrhage, nervousness, fading of body colour, anorexia, poor growth (6)
I. punctatus	Anorexia, poor growth, dark colouration, mortality (7-8)
P. major	Anorexia, poor growth (9)
A. anguilla	Anorexia, poor growth, ataxia, trunk winding syndrome, fin haemorrhage (10-11)
O. mossambicus x urolepis hornorum	Anorexia, light colouration, nervous disorders, poor feed efficiency and growth, low haematocrit (12)
L. calcarifer	Anorexia, dark colouration, poor growth, post handling shock, mortality (13)
FOLIC ACID	
Salmonids	Macrocytic normochromic anaemia, poor growth, anorexia, lethargy, dark colouration, pale gills, exophthalmia, distended abdomen with ascites fluid (1-5)
I. punctatus	Anorexia, increased mortality, lethargy, reduced growth, low haematocrit (7, 14)
A. japonica	Anorexia, poor growth, dark colouration (10)
Labeo rohita	Reduced growth and haematocrit (15)
C. batrachus	Anorexia, reduced growth, fading of body colour, pale gills and liver (16)

1/ 1-McLaren et al. (1947), 2-Phillips & Brockway (1957), 3-Halver (1957), 4-Kitamura et al. (1967), 5-Coates & Halver (1958), 6-Aoe et al. (1969), 7-Dupree (1966), 8-Murai & Andrews (1978), 9-Yone (1975), 10-Arai, Nose & Hashimoto (1972), 11-Hashimoto, Arai & Nose (1970), 12-Lim, Leamaster & Brock (1991), 13-Boonyaratpalin & Wanakowat (1991), 14-Duncan & Lovell (1991), 15-John & Mahajan (1979), 16-Butthep, Sitasit & Boonyaratpalin (1985)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
PYRIDOXINE (vitamin B6)	
Salmonids	Nervous disorders, hyperirritability, anorexia, rapid onset of rigor mortis, ataxia, oedema of peritoneal cavity, excessive flexing of opercules, erratic and rapid swimming, greenish-blue colouration of skin, anaemia, rapid and gasping breathing (1-10)
C. carpio	Anorexia, poor growth, nervous disorders (11)
I. punctatus	Anorexia, nervous disorders, erratic swimming, opercule extension, tetany, blue-green colouration of dorsal surface (12-13)
C. major	Poor growth (14)
A. japonica	Anorexia, poor growth, nervous disorders (15)
S. maximus	Reduced growth (16)
Sparus auratus	Anorexia, poor growth, high mortality, hyper-irritability, erratic swimming, poor feed efficiency (17)
S. quinqueradiata	Reduced growth (18)
Channa punctata	Reduced growth, ataxia, hyperirritability, muscular spasms, anorexia, erratic swimming, scale loss, oedema, abnormal pigmentation, lens opacy and blindness (19)
C. batrachus	Poor growth, increased mortality, eroded barbels, nervous disorders, loss of equilibrium, rapid onset of rigor mortis, erratic swimming, eroded fins and lower jaw, rapid breathing (20)
L. calcarifer	Anorexia, reduced growth, surface swimming, avoidance of schooling, erratic spiral swimming, lesions of lower lip, high mortality, convulsions, reduced food conversion ratio (21)

1/ 1-McLaren et al. (1947), 2-Phillips & Brockway (1957), 3-Halver (1957), 4-Kitamura et al. (1967), 5-Coates & Halver (1958), 6-Jurss (1978), 7-Hardy, Halver & Brannon (1979), 8-Jurss & Jonas (1981), 9-Smith, Brin & Halver (1974), 10-Herman (1985), 11-Ogino (1965), 12-Dupree (1966), 13-Andrews & Murai (1979), 14-Yone (1975), 15-Arai, Nose & Hashimoto (1972), 16-Adron, Knox & Cowey (1978), 17-Kissil et al. (1981), 18-Sakaguchi, Takeda & Tange (1969), 19-Agrawal & Mahajan (1983), 20-Butthep, Sitasit & Boonyaratpalin (1985), 21-Wanakowat et al. (1989)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
ASCORBIC ACID (vitamin C)	
Salmonids	Reduced growth, impaired collagen formation, scoliosis, lordosis, internal/fin haemorrhage, dark colouration, distorted/twisted gill filaments, poor wound repair, increased mortality, reduced egg hatchability (1-12)
I. punctatus	Reduced growth, scoliosis, lordosis, increased disease susceptibility, broken back syndrome, internal and external haemorrhage, fin erosion, dark skin colour, anorexia, erratic swimming behaviour (13-21)
C. major	Reduced growth (22,38), high mortality (38)
A. japonica	Reduced growth, fin/head erosion, lower jaw erosion (23)
C. punctata	Scoliosis, lordosis, anaemia, distorted gill filaments (24)
Tilapia	Scoliosis, lordosis, reduced growth/wound repair, internal/external haemorrhage, caudal fin erosion, exophthalmia, anaemia, reduced egg hatchability (25-26)
C. batrachus	Scoliosis, external haemorrhage, fin erosion, dark skin colouration (27)
Cirrhina mrigala	Reduced growth, increased mortality, scoliosis, lordosis, hypochromic macrocytic anaemia (28)
S. maximus	Reduced growth, renal granuloma, mortality (29-31, 37)
Pleuronectes platessa	Reduced growth and survival (32)
L. calacrifer	Reduced growth, dark colouration, loss of equilibrium, caudal fin erosion, haemorrhagic gills, short operculum, short snout, exophthalmia, short body , fragile gill filaments (33), club-shaped gill lamellae, fatty degeneration of liver, muscle degeneration, skin haemorrhage (39)
Sparus auratus	Renal granuloma (34)
C. urophthalmus	Reduced growth, high mortality, dark colouration, short operculae, haemorrhagic eyes, head and fins, erosion of the skin and fins, loss of scales, exophthalmia, swollen abdomen, scoliosis, lordosis, iritis, and changes in head bones (35)

Dicentrarchus labrax	Scale loss, dark colouration, emaciation, blindness, surface swimming, lower lip ulceration, increased mortality, scoliosis (vertebral column fractures) (36)
Sciaenops ocellatus	Increased mortality, scoliosis (vertebral column fractures), dark colouration (caudal region) (36)
Lutjanids	Scale loss, dark colouration, emaciation, blindness, surface swimming, lower lip ulceration (36)

1/ 1-McLaren et al. (1947), 2-Kitamura et al (1965), 3-Hilton, Cho & Slinger (1978), 4-Sato, Yoshinaka & Ikeda (1978), 5-Poston (1967), 6-Halver, Ashley & Smith (1969), 7-Sandnes et al (1984), 8-Navarre & Halver (1989), 9-Lall et al (1989), 10-Sato, Hatano & Yoshinaka (1991), 11-Dabrowski et al. (1990), 12- Cho & Cowey (1991), 13-Lovell (1973), 14-Andrews & Murai (1974), 15-Lovell & Lim (1978), 16-Wilson & Poe (1973), 17-Lim & Lovell (1978), 18-Li & Lovell (1985), 19-Mazik, Brandt & Tomasso (1987), 20-Lovell & Naggar (1989), 21-Wilson, Poe & Robinson (1989), 22-Yone (1975), 23-Arai, Nose & Hashimoto (1972), 24-Mahajan & Agrawal (1979), 25/26-Soliman, Jauncey & Roberts (1986, 1986a), 27-Butthep, Sitasit & Boonyaratpalin (1985), 28-Agrawal & Mahajan (1980), 29-Baudin Laurencin, Messager & Stephan (1989), 30-Coustans et al. (1990), 31-Gouillou, Coustans & Guillaume (1991), 32-Rosenlund et al. (1990), 33-Boonyaratpalin, Unprasert & Buranapanidgit (1989), 34-Paperna (1987), 35-Chavez de Martinez (1990), 36-Gallet de Saint Aurin, Raymond & Vianas (1989), 37-Messager et al. (1986), 38-Kanazawa et al (1992), 39-Boonyaratpalin, Boonyaratpalin & Supamataya (1992)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
CYANOCOBALAMIN (vitamin B12)	
Salmonids	Anorexia, reduced growth, microcytic hypochromic anaemia, fragmented erythrocytes, poor feed efficiency, dark pigmentation (1-2)
I. punctatus	Reduced growth, low haematocrit (3-4)
A. japonica	Reduced growth (5)
C. major	Reduced growth (6)
L. rohita	Reduced growth, low haematocrit, megaloblastic anaemia (7)
INOSITOL (Myo-Inositol)	
Salmonids	Reduced growth, distended abdomen, dark colour, increased gastric emptying time (1,8-10)
C. carpio	Reduced growth, skin and fin lesions/haemorrhage, loss of skin mucosa (11)
C. major	Reduced growth (6)
A. japonica	Anorexia, reduced growth, grey-white intestine (5)

1/ 1-Halver (1957), 2-Phillips et al. (1963), 3-Dupree (1966), 4-Limsuwan & Lovell (1981), 5-Arai, Nose & Hashimoto (1972), 6-Yone (1975), 7-John & Mahajan (1979), 8-McLaren et al. (1947), 9-Phillips & Brockway (1957), 10-Coates & Halver (1958), 11-Aoe & Masuda (1967)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
CHOLINE	
Salmonids	Reduced growth, fatty liver, poor feed efficiency, haemorrhagic kidney and intestine (1-8)
C. carpio	Reduced growth, fatty liver (9)
I. punctatus	Reduced growth, enlarged liver, haemorrhagic kidney and intestine (10-11)
A. japonica	Anorexia, reduced growth, grey-white intestine (12)
C. major	Reduced growth, mortality (13-14)
Acipencer transmontanus	Reduced growth, diffused fat vacuolation and fatty cyst formation in liver (15)
VITAMIN A (Retinol)	
Salmonids	Reduced growth, exophthalmia, depigmentation, clouding and thickening of corneal epithelium, degeneration of the retina (4-5,16)
C. carpio	Anorexia, faded body colour, fin and skin haemorrhage, exophthalmia, abnormal/warped gill operculae (17)
I. punctatus	Depigmentation, opaque and protruding eyes (exophthalmia), oedema, atrophy, kidney haemorrhage, increased mortality (10)
Poecilia reticulata	Reduced growth, poor feed efficiency, high mortality (18)
VITAMIN D_3 (Cholecalciferol)	
Salmonids	Reduced growth and feed efficiency, anorexia, tetany, elevated liver/muscle lipid content (19-20)
O. niloticus x O. aureus	Reduced growth and feed efficiency, low haemoglobin and hepatosomatic index (21)
I. punctatus	Reduced growth (22-24)

1/ 1-McLaren et al. (1947), 2-Phillips & Brockway (1957), 3-Halver (1957), 4-Kitamura et al (1967), 5-Coates & Halver (1958), 6-Ketola (1976), 7-Poston (1990), 8-Rumsey (1991), 9-Ogino et al. (1970a), 10-Dupree (1966), 11-Wilson & Poe (1988), 12-Arai, Nose & Hashimoto (1972), 13-Yone (1975), 14-Yano et al (1988), 15-Rumsey (1991), 16-Poston et al. (1977), 17-Aoe et al. (1968), 18-Shim & Tan (1989), 19-Barnett, Cho & Slinger (1979), 20-Leatherland et al. (1980), 21-Shiau & Hwang (1992), 22-Lovell & Li (1978), 23-Andrews, Murai & Page (1980), 24-Brown (1988)

Table 8. Reported dietary vitamin deficiency signs (continuation)

Vitamin/fish sp.	Deficiency signs [1/]
VITAMIN E (Tocopherol)	
Salmonids	Reduced growth, exophthalmia, ascites, anaemia, clubbed gills, epicarditis, ceroid deposition in spleen, increased mortality, pale gills, erythrocyte fragility, muscle damage/degeneration, reduced egg hatching rate/spawning efficiency, reduced antibody response (1-6)
C. carpio	Muscular dystrophy, mortality, exophthalmia (7-8)
I. punctatus	Reduced growth and feed efficiency, exudative diathesis, muscular dystrophy, depigmentation, fatty liver, anaemia, atrophy of pancreatic tissue, mortality, ceroid deposition in liver/blood vessels, splenic haemosiderosis (9-12)
O. niloticus/aureus	anorexia, reduced growth, poor feed efficiency, skin and fin haemorrhage, muscle degeneration, impaired red blood cell production, ceroid deposition in liver and spleen, lack of skin colour, increased mortality (13-14)
VITAMIN K_3 - MENADIONE	
Salmonids	Increased blood clotting time, anaemia, haemorrhagic gills, eyes, and vascular tissue (15-16)
I. punctatus	Skin haemorrhage (9-10)

1/ 1-Woodall et al. (1964), 2-Poston (1965), 3-Poston, Combs & Leibovitz (1976), 4-Cowey et al. (1984), 5-Ndoye et al. (1989), 6-Hardie, Fletcher & Secombes (1991), 7-Watanabe et al. (1970), 8-Watanabe & Takashima (1977), 9-Dupree (1966), 10-Murai & Andrews (1974), 11-Lovell, Miyazaki & Rabegnator (1984), 12-Wilson, Browser & Poe (1984), 13-Satoh, Takeuchi & Watanabe, (1987), 14-Roem, Stickney & Kohler (1990), 15-Poston (1964), 16-Poston (1976a)

Under intensive culture conditions, and in the absence of natural food organisms, dietary vitamin deficiencies may arise through one or more of the following:

Feed processing and storage

(i) **Riboflavin:** Slightly soluble in water and used in the form of a free flowing powder, riboflavin is generally stable in dry multivitamin premixes during extended storage and when mixed with mineral premixes and other feed ingredients. Processing losses of 26% have been reported for expanded pet foods (NRC, 1983). However, feeds containing riboflavin should be protected from intensive light/ultraviolet radiation (liable to oxidation) and alkaline conditions.

(ii) **Pantothenic acid:** Readily soluble in water and used in the form of calcium d-pantothenate (92% activity; solubility of ca. 40g/100ml) or calcium dl-pantothenate (46% activity), pantothenic acid is fairly stable in air and light if protected from humidity, but is sensitive to heat. Processing losses during pelleting or expansion have been reported to be as high as 10% within manufactured fish feeds (Slinger, Razzaque & Cho, 1979).

(iii) **Niacin:** Used in dried form as nicotinic acid or niacinamide, niacin is generally stable in dry multivitamin premixes and in the presence of air, heat and minerals. Processing losses of 20% have been reported for expanded pet foods (NRC, 1983).

(iv) **Thiamine:** Hydrochloride salt is freely soluble in water (ca. 1g/ml) whereas mononitrate salt is only sparingly soluble (ca. 2.7g/100ml). Thiamine hydrochloride is relatively stable to air if protected from light and humidity. Thiamine mononitrate is fairly stable to air if protected from light and is less sensitive to humidity than thiamine hydrochloride. Thiamine is usually stable in dry multivitamin premixes that contain no added choline or trace elements but is rapidly destroyed under alkaline conditions or in the presence of sulphide. Processing (pelleting/expansion) and storage (7 months, room temperature) losses of manufactured fish feeds have been reported to be 0-10% and 11-12%, respectively (Slinger, Razzaque & Cho, 1979).

(v) **Pyridoxine:** Readily soluble in water (ca. 20g/100ml) and used in dried form as pyridoxine hydrochloride. Pyridoxine is fairly stable to air and heat if protected from light and humidity, and is stable in dry multivitamin premixes that contain no added trace elements. Processing and storage (10 months) losses in manufactured fish feeds have been reported to be 7-10% (Slinger, Razzaque & Cho, 1979).

(vi) **Biotin:** Soluble in water and used in the form of d-biotin in dry dilution, biotin is fairly stable to air and heat in dry multivitamin premixes but sensitive to light and high humidity. Processing losses in expanded feeds have been reported as 10% (NRC, 1983).

(vii) **Folic acid:** Slightly soluble in water and used in dry-dilution, folic acid is fairly stable to air but is sensitive to heat and in particular to light and ultraviolet radiation. Processing and storage losses within manufactured fish feeds have been reported to be 3-10% (Slinger, Razzaque & Cho, 1979). There is some evidence to suggest that folate degrading bacteria are responsible for the destruction of folic within stored catfish feeds and that their actions are related to the occurrence of a nutritionally related anaemia in culture channel catfish (Plumb, Liu & Butterworth, 1991).

(viii) **Vitamin B12:** Moderately soluble in water and used in dry-dilution, vitamin B_{12} stability in multivitamin premixes is good at normal storage temperatures; elevated temperatures reducing activity, particularly under mild acid conditions.

(ix) **Choline:** Used in liquid (70% activity) or dried form (25-60% activity), choline chloride is stable in multivitamin premixes but can decrease the stability of other vitamins present, and therefore should be added separately. Relatively stable on processing and storage (NRC, 1983).

(x) **Vitamin C:** Crystalline L-ascorbic acid (AA) is readily oxidized and destroyed in the presence of oxygen, moisture, trace elements, elevated temperatures, light and oxidized lipids. It follows therefore, that considerable losses of vitamin C can occur during feed manufacture and on prolonged feed storage. For example, processing and storage losses for practical fish feeds have been reported to be as high as 95% for L-ascorbic acid (Slinger, Razzaque & Cho, 1979; Sandnes and Utne, 1982; Soliman, Jauncey & Roberts, 1987). However, the oxidation of vitamin C can be reduced by utilizing coated or 'protected' forms of L-ascorbic acid (ie. ethyl cellulose, silicone, gelatin, glyceride or synthetic-polymer coated ascorbic acid) or by using more stable biologically equivalent derivatives such as ascorbate-2-monophosphate (AMP) and ascorbate-2-polyphosphate (APP; Halver et al., 1975; Hilton, Cho & Slinger, 1977; Soliman, Jauncey & Roberts, 1987; Shigueno & Itoh, 1988; Grant et al, 1989; Lovell & Naggar, 1989; Skelbaek et al, 1990; Maugle, Brown & Hoffman, 1991; Halver, Felton & Palmisano, 1991; Sandnes, 1991). For example, losses of ascorbate activity from catfish diets containing AA or ethyl cellulose (EC) coated AA was reported to be 23-34% (AA) and 10-24% (ECAA) after conventional steam pelleting and 55-69% (AA) and 40-55% (ECAA) after expansion pelleting, respectively (Lovell & Lim, 1978). Although ECAA is generally regarded as being more stable than AA, Sandnes and Utne (1982) reported a 70-80% loss of ascorbate activity

with ECAA supplemented diets after steam pelleting and storage at 4°C for 24 weeks, while practically no activity could be detected after 16 weeks when the feed was stored at room temperature. By contrast, a 80-100% ascorbate recovery has been reported for APP within expansion pelleted catfish feeds (Lovell & Naggar, 1989); the stability of APP within feeds at 25 or 40°C being 83 or 45 times greater than that of AA, respectively (Grant et al, 1989).

(xi) **Vitamin A:** Normally used as the acetate, palmitate or propionate ester, in powdered or stabilized beadlet form. Although vitamin A is insoluble in water and stable within dry multivitamin premixes, it is readily oxidised at elevated storage temperatures and in the presence of oxidation products (rancid oils). Processing and storage losses of 20% and 53% have been reported for expanded pet foods and after six months storage at room temperature, respectively (NRC, 1983).

(xii) **Vitamin D_3:** Insoluble in water, vitamin D_3 is usually added in a protected beadlet form with vitamin A or as a dried powder. Stability is generally high within multivitamin premixes, during feed processing, and on storage.

(xiii) **Vitamin K_3:** Used in the form of the water soluble synthetic salt as menadione sodium bisulphite, the stability of vitamin K_3 in multivitamin premixes is good if protected from trace elements, heat, moisture and light (NRC, 1983).

(xiv) **Vitamin E:** Insoluble in water and used in the form of dl-alpha-tocopherol acetate, either spray dried or absorbed, vitamin E is moderately stable in dry multivitamin premixes if stored below room temperature. However, vitamin E is prone to oxidation on storage in the presence of oxidation products such as rancid oils and at high ambient temperatures.

(xv) **Inositol:** Myo-inositol is fairly stable within multivitamin premixes and to normal manufacturing and storage conditions.

For a review of the effects of processing and storage on vitamin stability see Coelho (1991) and Table 9.

Table 9. The effect of environmental factors on vitamin stability (after Coelho, 1991)

Vitamin	M 1/	O 2/	R 3/	T 4/	H 5/	L 6/	A 7/	N 8/	B 9/
A (beadlet)	S 10/	S	R	S	MS	MS	S	R	R
D (beadlet)	S	S	R	S	MS	MS	S	R	R
E acetate	R	R	R	MS	R	R	MS	R	S
K (MSBC, MPB) 11/	VS	R	MS	VS	MS	S	MS	R	S
Thiamine HCL	S	S	S	MS	S	R	R	MS	S
Thiamine mononitrate	R	MS	MS	MS	MS	R	R	MS	S
Riboflavin	R	R	MS	R	R	MS	R	MS	S
Pyridoxine	R	R	R	MS	R	S	R	MS	S
Vitamin B12	R	MS	S	MS	MS	S	MS	R	MS
Ca pantothenate	S	R	R	R	MS	R	S	MS	R
Folic acid	R	MS	MS	S	MS	MS	S	R	MS
Biotin	R	R	R	R	S	R	MS	R	R
Niacin	R	R	R	R	R	R	R	R	R
Niacinamide	S	R	R	R	R	R	MS	R	MS
Ascorbic acid	R	MS	R	VS	R	MS	R	R	S
Choline chloride	VS	R	R	R	R	R	R	R	MS

1/ M - Moisture, 2/ O - Oxidation, 3/ R - Reduction, 4/ - Trace minerals, 5/ H - Heat
6/ L - Light, 7/ A - Acid Ph, 8/ N - Neutral pH, 9/ B - Basic pH
10/ R - Resistant, MS - Mildly Sensitive, S - Sensitive, VS - Very Sensitive
11/ MSBP - Menadione Sodium Bisulphite Complex, MPB - Menadione Dimethyl Pyrimidinol bisulphite

Leaching of water soluble vitamins

In contrast to the fat soluble vitamins (A, D, E and K) the water soluble vitamins can be readily lost from the feed through leaching prior to ingestion by the fish. In general, the smaller the feed particle size and the longer the feed remains uneaten in water, the greater the loss of water soluble nutrients.

L-ascorbic acid (vitamin C) has been found to be particularly prone to loss through leaching. For example, despite the excessive losses of vitamin C which occur during feed preparation and storage (90-95% loss; Slinger, Razzaque & Cho, 1979), up to 50-70% of the residual vitamin C activity was found to be lost through leaching after a 10 second immersion period in water (1.18-2.36mm diameter pellet). In the same study, the authors also reported a 5-20% loss in pantothenic acid, 0-27% loss in folic acid, 0-17% loss in thiamine and a 3-13% loss in pyridoxine activity through leaching, after a 10 second water immersion period. Murai and Andrews (1975) reported a 50% loss in pantothenic acid after a 10 second immersion of a trout pellet originally containing 500 mg/kg pantothenic acid. Similarly, water stability tests with complete pelleted diets for shrimp (Penaeus japonicus) reported dietary vitamin losses through leaching of thiamine - 98% (initial 29.5 mg/kg), pantothenic acid - 94% (initial 100 mg/kg, as calcium salt), pyridoxine - 93% (initial 14 mg/kg), ascorbic acid - 89% (initial 3089 mg/kg), riboflavin - 86% (initial 55 mg/kg), nicotinic acid - 86% (initial 120 mg/kg), inositol - 52% (initial 4000 mg/kg) and choline - 45% (initial 3368 mg/kg, as chlorohydrate) after a one hour immersion period in seawater (Cuzon, Hew & Cognie, 1982).

Deficiencies due to the presence of dietary anti-vitamin factors

(i) An anti-biotin factor (avidin) is present in raw egg white, but is readily destroyed by heat.

(ii) Anti-thiamine factor (thiaminase enzyme) present in certain raw fish, shellfish, rice polishings, Indian mustard seed, mung bean (green gram), and linseed (Liener, 1980). The effect of thiaminase may be overcome by heat processing the raw material so as to deactivate the enzyme, or by using supplemental dibenzoylthiamine (DBT) as a thiaminase resistant form of dietary thiamine.

(iii) Anti-vitamin A, E, D and B_{12} factors present in raw soybean. May be deactivated by heat treatment (Liener, 1980).

(iv) Anti-pyridoxine factor present in linseed; treatment as above.

Deficiencies due to dietary antibiotic addition

The use of feed antibiotics for disease treatment may reduce the vitamin synthesising capacity of the gut micro-flora of fish, which in herbivorous/omnivorous fish species is thought to significantly contribute toward the vitamin requirements of the fish (carp, tilapia, channel catfish - vitamin B_{12}, folic acid and possibly biotin, thiamine and vitamin K; Lovell & Limsuwan, 1982; Lovell & Buston, 1984; Sugita, Miyajima & Deguchi, 1990).

Vitamins - stress and immunocompetence

There is some evidence to suggest that the dietary vitamin requirement of fish may be higher under 'stressed' or adverse environmental conditions (ie. vitamin C - Lovell & Lim, 1978; Hilton, 1989; Sandnes, 1991) and for increased immunocompetence and disease resistance (ie. vitamins C, E and possibly vitamin B_6 , pantothenic acid and choline - Hardie , Fletcher & Secombes, 1991; Ndoye et al., 1989; Blazer, 1991; Albrektsen et al, 1991; Lall, 1991; Landolt, 1989; Yano et al, 1988; Navarre & Halver, 1989; Waagbo et al, 1991; Verlhac et al, 1991; Sandnes, Rosenlund & Waagbo, 1991). However, considerable further work is required in this newly emerging field before these results (if reconfirmed under practical farming conditions) can be applied to the commercial fish farming community.

5.2 Dietary vitamin toxicity

In contrast to the water soluble vitamins, fish accumulate fat-soluble vitamins (A, D, E & K) under conditions where dietary intakes exceeds metabolic demand. Under certain circumstances accumulation is such that a toxic condition (hypervitaminosis) may be produced. Although such a condition is unlikely to occur under practical farming conditions, hypervitaminosis has been experimentally induced in fish and the reported toxicity signs shown in Table 10.

Table 10. Reported dietary vitamin toxicity signs in fish

Vitamin/fish sp.	Toxicity signs [1/]
VITAMIN A	
Salmonids	Reduced growth and haematocrit, severe necrosis/erosion of anal, caudal, pelvic and pectoral fins, scoliosis, lordosis, increased mortality, pale yellow livers (Hilton, 1983; Poston et al, 1966; toxic level of vitamin A 2.2-2.7 million I.U./kg diet)
VITAMIN D	
Salmonids	Reduced growth, lethargy, dark colouration (Halver, 1980)
I. punctatus	Reduced growth, poor feed efficiency (Andrews, Murai & Page, 1980). However, Brown (1988) reported no toxic effect up to 1 million I.U./kg)
VITAMIN E	
Salmonids	Reduced blood erythrocyte concentration (5000 mg of dl-α-tocopherol per kg of diet; Poston & Livingston, 1969)

6. ENDOGENOUS ANTI-NUTRITIONAL FACTORS PRESENT IN PLANT FEEDSTUFFS

The presence of endogenous anti-nutritional factors within plant feedstuffs is believed to be the largest single factor limiting their use within compounded animal and fish feeds at high dietary levels. Table 11 summarizes the major groups of anti-nutritional factors present in plant feedstuffs with more specific examples given in Table 12. Although these factors vary in their individual toxicity to fish, a large proportion of them can be destroyed or inactivated by heat treatment processes (Tacon & Jackson, 1985).

Unfortunately toxicological studies have not been performed on the majority of these anti-nutritional factors; on a general basis however their presence in untreated foodstuffs normally results in anorexia, reduced growth and poor feed efficiency when used at high dietary concentrations. For review see NRC (1983), Hendricks & Bailey (1989) and Lovell (1989).

Table 11. Classification of endogenous toxic factors occurring in plant foodstuffs of agricultural importance according to chemical properties (adapted after Liener, 1975)

PROTEINS	PROTEASE INHIBITORS HAEMAGGLUTININS
GLYCOSIDES	GOITROGENS CYANOGENS SAPONINS ESTROGENS
PHENOLS	GOSSYPOL TANNINS
MISCELLANEOUS	ANTI-MINERALS ANTI-VITAMINS ANTI-ENZYMES FOOD ALLERGENS MICROBIAL/PLANT CARCINOGENS TOXIC AMINO ACIDS

Table 12. Endogenous anti-nutritional factors present in plant foodstuffs commonly used for animal feeding 1/

Feedstuff	Anti-nutritional factor 2/
CEREALS	
Barley (Hordeum vulgare)	1,2,5,8
Rice (Oryza sativum)	1,2,5,8,13
Sorghum (Sorghum bicolor)	1,4,5,7,18
Wheat (Triticum vulgare)	1,2,5,8,11,18,22
Corn/maize (Zea mays)	1,5,8,19
ROOT TUBERS	
Sweet potato (Ipomoea batata)	1,19
Cassava (Manihot utilissima)	1,4
Potato (Solanum tuberosum)	1,2,4,8,18,19
LEGUMES	
Broad/faba bean (Vicia faba)	1,2,5,7,22
Chick pea/bengal gram (Cicer arietinum)	1,4,5,8,11
Cow pea (Vigna unguiculata)	1,2,5,11
Grass pea (Lathyrus sativus)	1,9
Haricot/kidney bean (Phaseolus vulgaris)	1,2,4,5,6,11,12,18
Hyacinth/field bean (Dolichus lablab)	1,2,4
Lentil (Lens culinaris)	1,2,6
Lima bean (Phaseolus lunatus)	1,2,4,5,7
Lupin (Lupinus albus)	1
Mung bean/green gram (Phaseolus aureus)	1,5,6,11,13
Field pea (Pisum sativum)	1,2,4,5,6,12
Pigeon pea/red gram (Cajanus cajan)	1,2,4,5
Rice bean (Vigna umbellata)	2
Runner bean (Phaseolus coccineus)	1,2
Sword/jack bean (Canavalia gladiata)	1,2,4,6
Velvet bean (Stizobolium deeringianuum)	1,22
Winged bean (Psophocarpus tetragonolobus)	1,2
Carob bean (Ceratonia siliqua)	1,7
Guar bean (Cyamopsis psoraloides)	1
Alfalfa/lucerne (Medicago sativa)	1,6,8,12
Black gram (Phaseolus mungo)	1,5
Ipil (Leucaena leucocephala)	23

OILSEEDS	
Groundnut (Arachis hypogaea)	1,2,5,6,8
Rapeseed (Brassica campestris napus)	1,3,5,7
Indian mustard (Brassica juncea)	1,3,13
Soybean (Glycine max)	1-3,5,6,8,11,12,14,16,17
Sunflower (Helianthus annuus)	1,7,20
Cottonseed (Gossypium spp.)	5,8,10,12,24
Linseed (Linum usitatissimum)	4,8,13,15
Sesame (Sesamum indicum)	5
Crambe (Crambe abyssinica)	3

1/ Compiled from the data of Kay (1979) and Liener (1980)

2/ 1-Protease inhibitor, 2-Phytohaemagglutinins, 3-Glucosinolates, 4-Cyanogens, 5-Phytic acid, 6-Saponins, 7-Tannins, 8-Estrogenic factors, 9-Lathyrogens, 10-Gossypol [a dietary level of 0.05% free gossypol suppresses growth and severely reduces blood haematocrit and haemoglobin in rainbow trout, a minimum of 0.03% causes growth depression, and 0.009% causes histological liver changes, including necrosis and ceroid deposition (Herman, 1970). By contrast, dietary gossypol levels of up to 0.18% were reported to be tolerated by Tilapia aurea without growth reduction (Robinson, Rawles & Stickney, 1984). However, according to Lovell (1989) the addition of 0.85 to 1.0 part of ferrous sulphate to each part of free gossypol in the diet of pigs and poultry has proven successful in blocking the toxic effects of gossypol], 11-Flatulence factor, 12-Anti-vitamin E factor, 13-Anti-thiamine factor, 14-Anti-vitamin A factor, 15-Anti-pyridoxine factor, 16-Anti-vitamin D factor, 17-Anti-vitamin B12 factor, 18-Amylase inhibitor, 19-Invertase inhibitor, 20-Arginase inhibitor, 21-Cholinesterase inhibitor, 22-Dihydroxyphenylalanine, 23-Mimosine, 24-Cyclopropenoic acid

7. ADVENTITIOUS TOXIC FACTORS PRESENT IN FEEDSTUFFS

Nutritional disorders and pathology may arise from the presence of specific adventitious toxic factors or contaminants within feedstuffs, including:

(i) Intentional additives
- binders and stabilizers (carboxymethyl cellulose, alginates, gums)
- therapeutic drugs (antibiotics, sulphonamides, nitrofurans, arsenilic acid)
- growth promotants (as above, plus anabolic steroids, synthetic androgens)

(ii) Toxic factors arising from processing
- solvent residues present in solvent extracted oilseeds (methylene chloride, ethylene dichloride, trichloroethylene, acetone, iso-propyl alcohol)
- lipids spoiled by oxidation and/or heat (rancidity, oxidation products)

(iii) Contaminants of biological origin
- protozoan toxins from spoiled fish
- algal toxins from shellfish/fish
- fungal toxins in stored feeds (ie. aflatoxins)
- bacterial toxins from contaminated feedstuffs (ie. botulinum toxin)
- pathogens (viable bacteria, viruses and fungi)

(iv) Synthetic contaminants
- pesticide residues (chlorinated hydrocarbons)
- organochlorine compounds (polychlorinated biphenyls)
- petroleum hydrocarbons
- heavy metals (mercury, cadmium, lead)

Of the above mentioned adventitious toxic factors probably the most important (from a pathological and economic viewpoint) are the fungal toxins or 'mycotoxins'. Over 200 different mycotoxins have been identified to date from feed ingredient sources (Jones, 1987). The majority of mycotoxins of pathological importance are produced by filamentous fungi (ie. moulds) belonging to the Aspergillus, Penicillium and Fusarium genera, and include the aflatoxins (aflatoxin B1-2, G1-2), Fusarium toxins (zearalenones, tricothecenes - vomitoxin, T2), ochratoxins (ochratoxin A & B), cyclopiazonic acid, patulin, slaframine, and citrinin (Hendricks & Bailey, 1989; Lovell, 1989, 1991, 1992). Mycotoxins are produced by the moulds within certain feedstuffs either prior to harvesting or during storage prior to animal consumption; feedstuffs which are particularly prone to attack by Aspergillus flavus (producer of the common mycotoxin aflatoxin B1 - AFB1) including cottonseed meal, groundnut meal, copra meal, maize (corn) products, and to a lesser extent wheat, rice, barley, sorghum, oats, sunflower, soybean and cassava (Hendricks & Bailey, 1989). For a review of the

factors favouring the growth of these contaminating moulds within feedstuffs see Tacon (1988). According to national feed legislation in the USA, maize (corn) and peanut (groundnut) products that are to be used for feeding dairy and immature animals (including fish) cannot contain more than 20 ppb of aflatoxin (Lovell, 1992).

Reported pathological signs of mycotoxin poisoning in fish include:
Aflatoxin: Fish general - poor growth, anaemia, impaired blood clotting, sensitivity to bruising, damage to liver and other organs, decreased immune responsiveness, and increased mortality. Prolonged feeding of a low concentration of aflatoxin (B1) to rainbow trout causes liver tumours (Lovell, 1992). Rainbow trout is reported to be one of the most sensitive animals to aflatoxin poisoning; the LD_{50} (dose causing death in 50% of the subjects) for aflatoxin in a 50g trout being 500-1000 ppb (0.5-1.0 mg/kg), and oral intakes of 0.4-1.0 ppb dietary AFB1 fed continuously for one year producing hepatic tumours (for review see Hendricks & Bailey, 1989). Signs of severe aflatoxicosis in rainbow trout include liver damage, pale gills and reduced red blood cell concentration. However, warm water fish such as channel catfish are reported to be less sensitive to aflatoxin; a dietary concentration of 6600 ppb aflatoxin B1 causing reduced growth rate, haematocrit and haemoglobin concentration in channel catfish over a 10-week trial period (Lovell, 1992). Coho salmon (O. kisutch), chinook salmon (O. tschawytscha) and sockeye salmon (O. nerka) are also reported to be considerably less sensitive to aflatoxin poisoning than rainbow trout (Hendricks & Bailey, 1989).
Ochratoxin A: Rainbow trout - severe necrosis of liver and kidney tissue, pale kidney, light swollen livers and death; LD^{50} of 4.67mg/kg (Hendricks & Bailey, 1989; Lovell, 1992).
Cyclopiazonic acid (CPA): Channel catfish - a dietary level of 100 ppb CPA significantly reducing growth, and 10,000 ppb causing necrosis of gastric glands. According to the above data CPA is more toxic to channel catfish than aflatoxin (Lovell, 1992).
Vomitoxin: Rainbow trout - a dietary level of 1-12.9 ppm causing reduced growth and feed efficiency (Hendricks & Bailey, 1989)

Apart from the mycotoxins, dietary toxicological studies have not been performed on the majority of the other above mentioned contaminants. For general review see NRC (1983), Hendricks & Bailey (1989) and Roberts & Bullock (1989).

8. CONCLUDING REMARKS

Table 13 summarises the dietary imbalances which have resulted in the manifestation of six major nutritional pathology conditions, namely 1) scoliosis/lordosis, 2) eye cataract, 3) fin erosion, 4) fatty liver, 5) exophthalmus, and 6) skin/fin haemorrhage. With the possible exception of the studies of researchers in the USA on the characterisation of eye cataracts in salmonid fish (Hughes, 1985;

Hargis, 1991; Kincaid & Calkins, 1991), there has been no systematic attempt to date to individually characterise the above mentioned conditions in fish. It is hoped that this review paper will stimulate further studies to be conducted under practical farming conditions so as to increase our understanding of nutritional fish pathology in fish.

Table 13. Major nutritional pathology conditions and reported causes

SCOLIOSIS/LORDOSIS	Deficiency of	Tryptophan Magnesium Phosphorus Vitamin C Essential fatty acids (lordosis)
	Toxicity of	Lead Cadmium Leucine Vitamin A Oxidized fish oil
CATARACT	Deficiency of	Methionine Tryptophan Zinc Magnesium Copper Selenium Manganese Vitamin A Riboflavin
	Toxicity of	Choline Oxidized fish oil
FIN EROSION	Deficiency of	Lysine Tryptophan Zinc Riboflavin Inositol Niacin Vitamin C
	Toxicity of	Lead Vitamin A
FATTY LIVER	Deficiency of	Choline Essential fatty acids
	Toxicity of	Oxidized fish oil

EXOPHTHALMIA	Deficiency of	Pantothenic acid Niacin Folic acid Vitamin A Vitamin E
	Toxicity of	Oxidized fish oil
FIN/SKIN HAEMORRHAGE	Deficiency of	Riboflavin Pantothenic acid Niacin Thiamine Inositol Vitamin C Vitamin A Vitamin K
	Toxicity of	Oxidized fish oil

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ANNEX

Scientific and Common Names of Species mentioned in this Report	
Species Name	**Common Name**
Acipenser transmontanus	Sturgeon
Anguilla japonica	Japanese eel
Channa (= Ophiocephalus) punctata	Snakehead
Cichlasoma urophthalmus	Mexican cichlid
Cirrhina mrigala	Indian major carp
Clarias batrachus	Walking catfish
Coregonus lavaretus	White fish
Ctenopharyngodon idella	Grass carp
Cyprinus carpio	Common carp
Dicentrarchus labrax	European seabass
Ictalurus punctatus	Channel catfish
Labeo rohita	Rohu
Lates calcarifer	Asian seabass
Oncorhynchus keta	Chum salmon
Oncorhynchus kisutch	Coho salmon
Oncorhynchus mykiss	Rainbow trout
Oncorhynchus nerka	Sockeye salmon
Oncorhynchus tshawytscha	Chinook salmon
Oreochromis mossambicus	Mozambique tilapia
Oreochromis niloticus	Nile tilapia
Pagrus major	Red sea bream
Pleuronectes platessa	Plaice
Poecilia reticulata	Guppy
Pseudocaranx dentex	Striped jack
Salmo salar	Atlantic salmon
Salvelinus fontinalis	Brook trout
Sciaenops ocellatus	Red drum
Scophthalmus maximus	Turbot
Seriola quinqueradiata	Yellow tail
Sparus auratus	Gilthead bream

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